Olewine

A Preliminary Study of the Composition of the Rhizome of the Black Cohosi Cimicifuga Racemosa

A PRELIMINARY STUDY OF THE COMPOSITION OF THE RHIZOME OF THE BLACK COHOSH CIMICIFUGA RACEMOSA

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY JAMES HARRIS OLEWINE

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A PRELIMINARY STUDY OF THE COMPOSITION OF THE RHIZOME OF CIMICIFUGA RACEMOSA.

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A Preliminary Study of the Composition of the Rhizome of the Black Cohosh, CIMICIFUGA RACEMOSA.

CIMICIFUGA RACEMOSA (Linne), Nuttal (Family RANUNCULACEAE), commonly known, among other names, as BLACK COHOSH, and BLACK SNAKEROOT, is a plant indigenous to North America, growing in shady or rocky woods from Canada to Florida, flowering in June and July. The roots of this plant, or preparations therefrom, are considerably employed in medicine, and the drug is at present recognized by the Pharmacopaeia of the U.S.A. It has been used in the past in "rheumatism", "dropsy", "hysteria", "phthisis", and various other affections, but at present is employed almost exclusively in the treatment of the "St. Vitus's Dance" of childhood, in which it is an efficient remedy.

CIMICIFUGA RACEMOSA appears to have been first chemically examined by John H. Tilghmann (1) who found fatty-matter, gum, starch, resin, tannin (and tannic acid), wax, gallic acid, sugar, an oil, black coloring matter, green coloring matter, lignin, and salts of lime, iron magnesium, and potassium. His experiments did not lead him to any decided conclusion as to the nature of the active principle of Cimicifuga.

Joshua S. Jones (2), from a series of experiments, believed the following to be the composition:- Lignin, gum, starch, tannin, resin, gallic acid, fatty matter, fixed oil, wax, black coloring matter, volatile oil, and salts of magnesium, potassium, iron and lime.

George H. Davis (3), from a series of experiments, believed the

following to be the composition:- Albumen, uncrystallizable sugar, tannic acid, gallic acid, gum, extractive, starch, resin soluble in alcohol and insoluble in ether, resin soluble in alcohol and soluble in ether, fatty matter, waxy matter, volatile oil, green and brown coloring matters, lignin, and salts of potassium, magnesium, line and iron, (and Silica).

A crystallizable principle has been obtained by T. Elwood Conard (4), from a strong tincture of the root by treating with solution of lead-subacetate, which precipitated resin, tannin, and coloring matters, then filtering, and precipitating the lead by hydrogen sulfide in excess, and allowing the tincture to evaporate spontaneously; and, finally, having treated the residuary powder with petroleum benzene, afterwards, washing it with water, dissolving it to saturation in strong alcohol, and treating the solution with alumina. The mixture was allowed to evaporate to a dry mass, which was nearly exhausted with alcohol. The solution, being allowed to evaporate, left behind a crystalline mass, somewhat resembling alum. "This substance has little taste, on account of its extreme insolubility in saliva, but in alcoholic solution has very strongly the acrid taste characteristic of the fresh root. The crystals are very soluble in cold, and more so in hot alcohol. Soluble also in chloroform, and slightly so in ether. They are fusible and imflammable. They are neutral, possessing neither acid nor alkaline properties." Conard concluded that the crystallizable principle is neither an alkaloid nor an acid principle.

L. F. Beach (5), obtained from commercial resin of Cimicifuga (the so-called "Cimicifugin" or "Macrotin") a crystalline

principle resembling the principle announced by Conard. On the other hand, neither F. H. Trimble (6), nor Warder and Coblentz were able to obtain a crystalline principle, while C. S. Gallaher (7) obtained crystals of cane-sugar from the fluid-extract. J. U. and C. G. Lloyd dismiss the subject, after carefully weighing all the evidence, with the statement, "We are convinced the Cimicifuga does not contain a crystalline principle". (8) In view of these facts, it would appear that the active principle is a resinous amorphous body.

The purpose of this investigation was to determine the Composition of the Rhizome of the Black Cohosh, CIMICIFUGA RACEMOSA. The general scheme of analysis followed was a summary of the methods found in the published papers from the Wellcome Chemical Research Laboratories, with modifications suggested by Parsons, and by Dragendorff.

A condensed summary of Power's Scheme for Plant Analysis, as used in the Wellcome Laboratories, in general, and for this investigation in particular, is as follows:-

1. PRELIMINARY EXAMINATION.

   (a) Extract a small quantity of the plant with Prollius Solution and make the usual tests for Alkaloids.

   (b) Extract weighed quantities of the plant (usually 50 grams) in Soxhlet with the following solvents in the order named, weighing each extract dried at 110 degrees, Low-boiling petroleum ether, Ethyl ether, Chloroform, Ethyl acetate, and Ethyl alcohol.

2. ACTUAL ANALYSIS.

Extract the plant completely with 95% alcohol, either by percolation in the cold, or by treatment with hot alcohol in Soxhlet.

Evaporate the alcohol from the extract thus obtained, and treat the thick extract by one of the following schemes:-

(1) Mix with water and distil with steam, obtaining the steam distillate (A) and the water-solution (B) and the residue (C).)B) and (C) are separated by filtering.

(2) The water-solution is sometimes separated hot, and on cooling, a second residue (resin), (C_1) is obtained. Distillate (A) contains volatile oils and other volatile substances. Extract (A) with ether, and shake the ethereal extract with the following succession of solvents:- (1) Strong solution of ammonium carbonate, (2) A 5% solution of sodium carbonate, and (3) 2-5% sodium hydroxide. (The first extracts the stronger acids, the second extracts the weaker acids and phenols, and the third, the weakest acids and phenols. These solutions are acidified, and the precipitates on examination are usually found to contain small amounts of fatty acids.)

Or, take the residues after extraction of (A) with ether, and extract with petroleum ether, ethyl ether, chloroform, and amyl alcohol, and ca ll the resulting solutions, B_1, B_2, B_3, and B_4, respectively.

Shake out each extract with ammonium carbonate, sodium carbonate, and sodium hydroxide. Treat (B) with barium hydroxide to precipitate water-soluble organic acids. Then add lead-acetate to precipitate tannins. Test the original solution with Fehling's solution and Phenylhydrazine for sugars.

Residue (C) contains the most material. Take up with alcohol, and then mix with purified sawdust (extract oak sawdust with hot alcohol). Extract in Soxhlet with petroleum ether, ethel ether, chloroform, ethyl acetate, and ethyl alcohol, and call the resulting extracts C_2, C_3, C_4, and C_5, respectively.

Extracts C_1, C_2, and C_3 are sometimes shaken with ammonium carbonate, sodium carbonate, and sodium hydroxide and HCl. The HCl removes basic substances. The petroleum and ethyl ether extracts are then evaporated and saponified with alcoholic KOH. Evaporate the alcohol and take up the soap with water. Shake with ether to remove the unsaponifiable matter (hydrocarbons and wax-alcohols).

Acidify with sulfuric acid, distil with steam to take out any volatile fatty acids and dissolve any nonfolatile fatty acids with ether.

Hydrolyze C_3, C_4, and C_5, and test for sugars and glucosides.

EXPERIMENTAL.

The material employed for this investigation consisted of an air-dried sample of the rhizome and roots of Black Cohosh, CIMICIFUGA RACEMOSA, collected by Dr. G. D. Beal during 1916 in the vicinity of Scio, Ohio.

The following preliminary tests were first made with a well-ground sample:-

Test for Alkaloid:-

A small sample (10 grams) was repeatedly shaken with 200ccs. of the cold Prollius' solution and filtered. The filtrate, when treated with acidulated water (1% HCl), gave no reactions for an alkaloid.

(The Prollius' solution consisted of the following:- 250 ccs. ethyl ether, 100 ccs. chloroform, 25 ccs. ethyl alcohol, and 10 ccs. concentrated ammonia.)

Test for Inorganic Constituents:-

Ignited in a porcelain crucible several grams of the sample to faint redness to free carbonaceous matter. In this residue determined:-

1. Water - soluble Salts.

Ca - $Ca'' + (NH_4)_2C_2O_4 = CaC_2O_4$

Mg - $Mg'' + Na_2HPO_4 + NH_4OH = MgNH_4PO_4$

Na - Na'' + alcohol + $H_2SiF_6 = Na_2SiF_6$ Flame test.

K - K' + alcohol + $HBF_4 = KBF_4$ Flame test.

2. Water - Insoluble; soluble in dilute HCl.

$$Fe''' - Fe''' + K_4Fe(CN)_6 = Fe_4(Fe(CN)_6)_3$$

3. Water-insoluble; acid and alkalia-insoluble.

Complex silicate. (A little unconsumed carbon remained after the above treatment.)

The statement of the analyses by Tilghmann, Jones and Davis, previously referred to, that this plant contains salts of lime, iron, magnesium, and potassium, (and silica), were thus confirmed.

Extraction with Water:-

A small sample (25 grams) was extracted with successive portions of hot water. A light reddish-brown filtrate was obtained, which frothed considerable on shaking, but the froth was not very persistent. The liquid gave no appreciable color-change with $FeCl_3$, but was precipitated by normal lead acetate and by lead subacetate. It gave no reduction of Fehling's solution, but after boiling with acid (HCl), it gave a slight reduction. The frothing of the original aqueous liquid resembled that produced when certain solutions of vegetable extracts containing gum are shaken. There was noticed, also, a substance in the alcohol-soluble resins, later on, that produced strong and persistent frothing when shaken with water. This was probably due to a substance of glucosidal character.

Extraction with Various Solvents:-

Another portion (50 grams) of the sample was successively extracted in a Soxhlet tube with the following solvents, and the extracts dried at 100 degrees were weighed, with the following results:-

Petroleum ether (low-boiling)	extracted	5.5%
Ethyl ether	"	1.1%
Chloroform	"	5.7%
Ethyl alcohol	"	13.1%

The petroleum ether solution was light yellowish-green in color and the evaporated extract was a reddish-brown oil, only sparingly soluble in cold alcohol, but readily soluble in hot alcohol, and separating on cooling.

The ether solution was yellowish in color, and the dried extract was resinous and light red in color. When warmed with a little water and filtered, the solution gave with $FeCl_3$ only a faint yellow coloration.

The chloroform solution was light brown, and the extract was resinous, and of a reddish-brown color. A filtered water solution of the extract gave only a very faint yellowish coloration with $FeCl_3$.

The alcoholic extract was dark brown, and of a resinous nature also. When taken up with a little hot water and filtered, the aqueous liquid gave a deep olive-green color with $FeCl_3$, and was colored bright yellow by alkalies (10% NaOH), and gave a strong reduction of Fehling's solution.

It is to be concluded, therefore, that principally the alcoholic extract is resinous, and that tannin as an intermediate product in resin formation was present in considerable quantities. The statement by Davis, previously referred to, that this plant contains green and brown coloring matters, was confirmed.

Extraction with 95% Alcohol:-

For the purpose of a complete examination of the constituents of the sample, 2.7 kilograms of the dried, 60-mesh material were extracted by continuous percolation with cold 95% alcohol. The percolate (16 liters) was concentrated under diminished pressure to a volume of 0.5 liters.

This extract was poured into 3 liters of distilled water with vigorous stirring. A heavy, sticky, straw-colored resinous material separated, but settled incompletely. The separation of the water-soluble from the water-insoluble portion was difficult. Standing, and the addition of electrolytes ($NaCl$, HCl, NH_4NO_3) did not help. Centrifuging was tried with little success, as was greezing, with similar results. But with an apparatus as described by R. C. Shuey (9(, wherein asbestos-pulp was employed, a clean-cut separation was effected. The 5 liters of water-soluble extract was dark green in color, but the clear filtrate became turbid on standing, due to oxidation. This could be clarified by filtration.

The same clarifying-effect on the water-soluble extract was effected by treatment once with ether. As was stated above, the aqueous liquid was turbid and did not afford a clear filtrate, owing apparently to a small amount of suspended resin. It was therefore shaken with ether, which rendered it perfectly clear. The ether solution was evaporated to dryness and the residue dissolved in alcohol. The alcoholic extract was concentrated and poured into water, when a small quantity of the previously described straw-colored resin was precipitated, which,

when dried, formed a dark brown amorphous mass, and was not further examined here.

(Following with continuous percolation with 50% alcohol, a much heavier percolate of 5 liters was obtained. Finally, 5 liters of 95% alcohol was used, when a percolate resembling the first one was obtained. These extracts were examined separately.)

After the above-described operations there remained the dark green aqueous liquid (A), and the yellowish resin (B).

EXAMINATION OF THE AQUEOUS LIQUID (A).

The aqueous liquid, clarified as above described, had a green color, a slightly bitter taste, and a slightly acid reaction. With $FeCl_3$, it gave a dark brown coloration, and with alkalies, a weakly yellow color. It readily reduced Fehling's solution.

After concentration under diminished pressure to a small volume, the aqueous liquid was shaken with successive portions of ether, when finally nothing seemed to dissolve. After the evaporation of the ether from a portion of the ethereal solution, a small quantity of a reddish-brown syrup was obtained. This was dissolved in hot alcohol, filtered, and the hot alcohol poured into a small quantity of boiling water. This aqueous liquid was filtered from a very small quantity of a yellowish resin that formed, evaporated to dryness, and the residue dissolved in ether. The evaporated ether extract was dark brown, and its color in chloroform was light green. It was readily soluble in water, and its aqueous solution would not reduce Fehling's solution. With $FeCl_3$ a light yellowish coloration was obtained. There was no assurance of this being an individual substance and the amount of material was too small to obtain anything definite from, but the indications point to an acidic substance with a coloring matter, perhaps chlorophyll indicated by the green coloring in chloroform.

The dry residue gave a dark brown color to concentrated H_2SO_4, and it readily dissolved with a yellowish color in a 10% solution of KOH, and was reprecipitated on the addition of the acid. A portion of this residue was treated with concentrated KOH and boiled. The dark brown melt was taken up in water, acidified with H_2SO_4, and distilled with steam. The acid filtrate was neutralized with Na_2CO_3, filtered, and evaporated, when it formed a yellowish syrup which could not be crystallized. The solution of this substance with a little alcohol and H_2SO_4, developed a doubtful odor of ethyl acetate. The volatile products of the treatment with KOH seemed to consist of acetic acid, but the indication was so delicate as to render the test doubtful. The acid residue from the steam distillation was saturated with ammonium sulfate and shaken out with ether. The ether was removed by evaporation, when a brown residue was obtained, from which nothing crystalline could be obtained. Its water solution gave a light brown color with $FeCl_3$.

Another portion of the original ethereal solution was shaken successively with 1% ammonium carbonate, 1% sodium carbonate, and 1% sodium hydroxide solutions. Each of these aklakine liquids was acidified with dilute sulfuric acid, extracted with ether, and the solvent evaporated. The material soluble in ammonium carbonate was small in amount, and consisted chiefly of a slightly yellow amorphous product. It seemed to contain, also, an acid soluble in warm water, which, after purification with charcoal, gave a bluish-green precipitate with $FeCl_3$. Lead acetate gave a bulky white precipitate,

turning reddish with 10% NaOH. Fehling's solution was turned from blue to yellowish-brown immediately. The material could not be purified sufficiently to run a melting-point, - crystallization from water and a variety of organic solvents failing. It would not sublime. When dissolved in NaOH, and neutralized with appropriate acids, the following salts were precipitated:-

HNO_3 + $AgNO_3$ gave a brownish-black precipitate at once.
HCl + $BaCl_2$ gave a white precipitate.
H_2SO_4 + $CaSO_4$ gave a white precipitate.

The above tests are not conclusive for any water-soluble organic acid, but certain indications point to the presence of gallic acid.

The sodium carbonate and sodium hydroxide yielded no information.

After the extraction of the original aqueous liquid with ether as above described, separate portions of it were treated as follows:-

Part 1 was shaken repeatedly with warm amyl alcohol. These amyl alcohol liquids were united, washed with water, concentrated under diminished pressure to the consistency of a syrup, and the last traces of amyl alcohol removed by passing steam thru the liquid. (A trace of reducing sugar was found in the washings.) A portion of the above mentioned syrupy product was heated for a few minutes with a 10% solution of KOH, the liquid then cooled, acidified with H_2SO_4, and extracted with ether. The ethereal liquid was afterward shaken with successive portions of aqueous 1% ammonium carbonate, sodium carbonate, and sodium hydroxide solutions, and the alkaline liquids

separately acidified with dilute sulfuric acid, and the ethereal extracts of these portions evaporated to a dryness. Slightly colored traces of amorphous products resulted in each case, but all efforts to crystallize anything from various organic solvents failed. The indications here point to the presence of a combination of water-soluble organic acids in the form of their esters, perhaps, but the amounts of material were too small to effect a separation and to obtain an indication of the separate acids present.

The remaining portion of the syrupy liquid was then treated with a solution of basic lead-acetate until no further precipitate was produced, the precipitate collected and thoroly washed with water, the washings being added to the main portion of the filtered liquid.

(The basic lead-acetate was made according to the directions given (10), which is:- "Boil for 30 minutes 430 grams of normal lead-acetate, 130 grams of PbO, and 1 liter of water. Cool the mixture, and allow to settle, and dilute the supernatent liquid to a specific gravity of 1.25 with recently distilled water".)

A portion of the above mentioned basic lead-acetate precipitate was suspended in water, decomposed by hydrogen sulfide, and the mixture filtered. The filtered liquid was then concentrated under diminished pressure to the consistency of a syrup. A portion of it gave a bluish coloration with $FeCl_3$, indicating an iron-bluing tannin, and was precipitated by a starch solution, and by an albumen solution. This indicates the presence of tannin (tannic acid), but no attempt was made to separate the gallic acid that certainly must have accompanied

the tannin. Another portion of the syrupy liquid that resulted from the decomposition of the basic lead-acetate precipitate by hydrogen sulfide, was neutralized with KOH and boiled for about 15 minutes. The liquid was then poured into dilute sulfuric acid, and, after cooling, the mixture was extracted many times with ether, the combined ethereal liquids being subsequently extracted with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide. The ammonium carbonate extract, after acidification, and extraction with ether, yielded a white amorphous substance that could not be crystallized from a variety of solvents. This substance would not sublime, and a molecular weight determination by the silver-salt method, indicated an acid with a molecular weight of approximately 500.

The pale-yellow filtrate from the precipitate produced by basic lead-acetate was treated with hydrogen sulfide for the removal of excess lead, again filtered, and concentrated under diminished pressure to the consistency of a syrup. A portion of this syrup gave a strong reduction of Fehling's solution, and responded to Molisch's color test. It evidently contained an abundance of sugar, since it readily yielded an osazone melting at 206 degrees. This must be phenylglucosazone. Another portion of the syrup boiled with 3% HCl, and treated again with Fehling's solution gave an increased reduction, indicating sucrose and glucosides in addition to the sugar found above. Another portion of the syrupy liquid deposited nothing on standing, and a portion of it was treated with acetic anhydride to see whether or not a crystalline acetyl derivative of the sugar could be prepared, - with a negative

result. Heated another portion with KOH to see if protein substances could be present to give ammonia fumes. Only a very slight indication, and slowly, showed that protein substances were probably absent. Tested other portion with I in KI, which gave a yellow precipitate of PbI_2, probably, and with $Hg(NO_3)_2$, which gave a slight turbidity. The remaining portion of the syrupy liquid was made alkaline with NaOH, and extracted successively with ether, chloroform, and warm amyl alcohol. Sugary amorphous products resulted which defied crystallization from a variety of solvents.

Part 2, to which no amyl alcohol was added, was treated with an excess of basic lead-acetate, when a heavy yellowish-white precipitate was obtained. This was filtered, and the precipitate washed with water. The precipitate was suspended in water, decomposed by hydrogen sulfide, and the liquid filtered. The filtrate had a very faint yellow color, and gave a faint green coloration with $FeCl_3$. It was extracted successively with ether, chloroform, ethyl acetate, and absolute alcohol. The residues resulting after the evaporation of the respective solvents were very small in amount, amorphous, and very slightly colored, from which nothing definite could be obtained. An alcoholic solution of all the residues separately gave a very faint green coloration with $FeCl_3$, indicating the presence of sugary residues.

EXAMINATION OF THE RESIN (B).

The water-insoluble resin (440 grams) which had been separated from the aqueous liquid (A), as previously described, was dissolved in ethyl alcohol to a volume of 2 liters, absorbed in purified oak sawdust, and the thoroughly dried mixture extracted successively by continuous percolation in Soxhlet with petroleum ether, ethyl ether, chloroform, ethyl acetate, and ethyl alcohol.

Petroleum ether Extract of the Resin.

This extract, after the removal of the solvent, was a light reddish-yellow oil when hot, and a yellowish-white soft solid when cold. It was dissolved in ether, and the ethereal solution extracted successively with 1% ammonium carbonate, sodium carbonate and sodium hydroxide. The clear sodium carbonate and sodium hydroxide liquids yielded, on acidification and extraction with ether, only very slight traces of a dissolved substance, while the ammonium carbonate yielded nothing. Nothing definite could be isolated from either of these extracts. Mean molecular weight determinations by titration with KOH indicated acidic substances of high molecular weight.

The ethereal liquid from which the alkalies had extracted nothing definite was then washed with water, and the solvent evaporated, when a small quantity of the previously described soft yellow solid was obtained. This fatty residue was dissolved in alcohol and refluxed for several hours with alcoholic KOH. After the removal of the greatest part of the solvent by a current of steam, the liquid was diluted with water,

and the resulting mixture extracted several times with ether. The solvent was removed from the combined ethereal extracts, when a small amount of a yellow, viscid solid was obtained. This was redissolved and reproduced several times from alcohol, but the material was insufficiently purified to obtain a melting-point with any degree of accuracy. The melting-points of successive deposits ranged from 65 to 120 degrees. The residue apparently consisted of a mixture of an alcohol and a hydrocarbon, but the amount was too small to permit of their separation.

This above mentioned unsaponifiable material was then distilled under diminished pressure, when most of it distilled with apparent decomposition, the final product being a viscid, yellow gum-like substance which did not crystallize on cooling. A little of this substance, when dissolved in a mixture of chloroform and acetic anhydride, and several drops of concentrated sulfuric acid subsequently added, gave a reddish-brown coloration, changing slowly to a light green. An attempt was made to crystallize this substance from alcohol, but no definite crystalline form could be detected, the amount of material apparently being too small to work with. The above-described substance was evidently a phytosterol, or some allied substance.

The alkaline aqueous liquid after the removal of the unsaponifiable substance by extraction with ether, was acidified with dilute sulfuric acid, and the precipitated fatty acids dissolved in ether. The ethereal solution was washed and evaporated, when a very small amount of a reddish-brown semi-solid residue was obtained. Here again fractional distillation

under diminished pressure was attempted, but everything distillable seemed to pass over in one fraction, which solidified rapidly but which could not be crystallized from a variety of solvents. It was converted into the lead salt, and the latter treated with ether, when a portion was insoluble, and the rest of it soluble. An attempt was made to crystallize the ether-insoluble portion, but nothing definitely crystalline could be obtained. Neutralization value with N/5 KOH were run on several minute portions of the residue with widely differing results, indicating the presence of impurities. One set of figures that came closest to the normal value for the common saturated acids was:-

.1058 grams of residue required 1.4 ccs. of N/5 KOH
(1cc. N/5 KOH being equivalent to 0.01844 grams of KOH)
(Lewkowitsch (11))
The neutralization value in this case was 229.5.
Since palmitic acid requires a neutralization value of 219.1 and stearic acid one of 197.5, the chances are that the fatty-acids above described is a mixture of palmitic or stearic, and acids of lower molecular weight.

The ether-soluble portion of fatty-acids was very much smaller in amount, barely a trace of the free fatty-acid resulting when the ethereal solution was treated with HCl to recover the free acid. But the presence of unsaturated acids was indicated by the reduction of alkaline permanganate.

Ether Extract of the Resin:-

This extract after the removal of the solvent, was a

dark reddish resinous substance. A portion of it was dissolved in ether, and the ethereal solution extracted successively with 1% ammonium carbonate, sodium carbonate, and sodium hydroxide. The clear alkaline liquids yielded, on acidification with sulfuric acid, and extraction with ether, no traces of a dissolved substance.

Another portion of the original resinous substance was sparingly soluble in alcohol, but dissolved readily in chloroform, and when acetic anhydride and several drops of concentrated sulfuric acid were added, it gave a pink coloration, slowly changing to greenish-yellow. From this it was concluded that the sparingly soluble substance was another portion of the higher alcohol member that appeared in the petroleum ether extract.

Chloform Extract of the Resin:-

This extract was very small in amount and was a light brown amorphous substance, and nothing was isolated from it.

Ethyl Acetate Extract of the Resin:-

This extract was slightly larger in amount than the chloroform extract, and was dark reddish-brown in color. To determine whether or not it contained anything glucosidic, it was heated for several hours with a 10% solution of sulfuric acid in aqueous alcohol solution. The aqueous, acid liquid was then shaken out with ether, which only removed a small amount of gummy-like material, and the sulfuric acid removed by means of barium hydroxide. The filtrate was then concen-

trated to a small volume and treated with Fehling's solution, when a heavy reduction was obtained. This would indicate a glucoside in the ethyl acetate extract of the resin.

Alcohol Extract of the Resin:-

This extract of the resin was a brownish-black amorphous substance, and was considerable in amount. When the test for a glucosidic material was applied as in the above extract with ethyl acetate, only a very faint reduction of Fehling's solution was noticed, which would indicate that the alcoholic extract of the resin was not distinctly glucosidic.

The following tests were then applied to the original sample of the well-ground root after continuous percolation with cold alcohol had apparently removed all that was soluble in that medium:-

Cold Water Extract:-

That part of the 2.7 kilograms sample insoluble in alcohol was dried and a small portion thoroughly extracted by cold water. (Allowed to macerate, with frequent stirring, for several days.) After filtering, the filtrate was light yellow in color, and the residue from the evaporation of a small portion was a yellowish, amorphous substance. Macerated this residue with alcohol and filtered. The evaporated alcoholic filtrate gave no residue, which indicated the absence of alcohol-soluble substances in the water-soluble extract.

The unevaporated water-soluble portion was tested qualitatively for the following substances, with the following

results:-

1. Gum and Dextrins:-

Basic lead-acetate gave a heavy white precipitate, which, if it was due to natural gums , would eliminate the possibility of dextrin or starch-gum from being present. (Dextrin being an artificial product obtained from starch.)

The original aqueous liquid did not reduce Fehling's solution, but prolonged boiling with concentrated sulfuric acid produced an hydrolysis, - the resulting solution giving a decided reduction of Fehling's solution.

Alcohol precipitated a white substance from the water-soluble solution.

Altho the evidence is not conclusive, the presence of a gum is indicated.

2. Pectin Bodies:-

As stated above, alcohol added to the water-soluble solution produced a double gelatinous complex, which might also indicate the presence of Pectin Bodies.

3. Lignin:-

On oxidation with nitric acid, another portion of the aqueous solution gave a substance the gas from which caused a slight turbidity in lime-water. This was probably due to carbon dioxide, which might have come from oxalic acid which might have been produced by the oxidation of the lignin by nitric acid.

Another portion of the concentrated extract gave with phloroglucinol and HCl a reddish coloration, perhaps indicating the presence of Lignin.

4. Organic Acids:-

Another portion of the aqueous solution when treated with HCl to liberate free organic acids, from their salts, gave precipitates with $BaCl_2$, and $AgNO_3$.

5. Mucilages and Higher Carbohydrates:-

Another portion of the aqueous liquid was mixed with absolute alcohol and allowed to stand several days in a freezing-mixture. The filtrate was examined before and after adding HCl with Fehling's solution. A positive reduction only after boiling with the acid indicated the hydrolysis of mucilages or higher carbohydrates.

As a further test for Lignin and Pectic Bodies, in the water-soluble extract, methylene - blue was added to the residue from the evaporated extract. A blue-black stain was produced, which, on treatment with alcohol and dilute acids gave no apparent decolorization. (Lignified bodies retain their coloration, while pectin bodies are decolorized.)

Acid Extract:-

The dried residue insoluble in water was transferred to a beaker containing water and concentrated sulfuric acid (100:1). Boiled several hours, adding water to keep the volume of the liquid constant. When filtered, the filtrate was reddish-brown in color. A negative Fehling's test before boiling, and a positive test after boiling, indicated the hydrolysis of starch and its amorphous isomers to glucose.

Calcium oxalate was precipitated by ammonium oxalate as a white crystalline precipitate from the acid-soluble extract.

Alkali Soluble:-

The dried residue insoluble in water and unchanged by H_2SO_4, was boiled for several hours with 20% $NaOH$, Filtered and evaporated the filtrate to obtain alkali-soluble substances. The nature of the residue indicated certain decomposition products.

Cellulose:-

The residue remaining after the above treatments, was macerated with Schqeitzer's Reagent, and filtered. Cellulose was reprecipitated from the filtrate as a yellowish-brown precipitate upon the addition of HCl.

(Schweitzer's Reagent.- Add NH_4Cl and then an excess of $NaOH$ to a solution of a cupric salt; the blue precipitate so obtained is then washed, pressed on a cloth filter, and dissolved in 0.92 ammonia).

SUMMARY AND CONCLUSIONS.

The experimental work described in the preceding pages suggests the following summary:

1. The rhizome of CIMICIFUGA RACEMOSA contains no alkaloid.
2. Beside gum and sugar, the roots contain salts of calcium, iron, potassium, sodium and magnesium and silica. There are also brown and green coloring matters.
3. The rhizome contains a considerable amount of tannin of the variety which give a bluish coloration with ferric chloride.
4. The water-soluble portions of the alcoholic extract gave indications of the presence of water-soluble organic acids, but the amount of material available were too small to permit of their separation and identification. A hydrolysis with KOH gave indications of the presence of acetic acid. The identification of phenylglucosazone after hydrolysis indicated the presence of considerable sucrose and glucosides.
5. The constituents of the resin soluble in petroleum ether, after hydrolysis with alcoholic potash, were as follows:

   (a). The portion of the hydrolyzed product which had entered into combination with the alkali yielded a very small amount of acidic substances. From a series

of determinations of the neutralization value there was no assurance of the fatty acid being present as individual substance, but the indications pointed to the presence of palmitic and stearic acids, and to an unsaturated fatty acid, the presence of which in a minute trace was indicated by reduction of alkaline $KMnO_4$.

(b) The portion of the hydrolyzed product which had not been extracted by the alkali was found to contain a small amount of an alcohol and a phytosterol, or some allied substance.

6. The resins soluble in chloroform and alcohol were amorphous substances, and not glucosidic.

7. The ethyl-acetate soluble-resin consisted principally of a glucosidic substance.

8. The portion of the rhizome insoluble after continuous percolation with 95% alcohol, gave indications for the following substances:- gums, lignin, pectin bodies, mucilages and higher Carbohydrates, starch and its isomers, and cellulose.

In addition, it might be stated that the amount of material employed in this investigation was too small to permit of the separation and identification of the various constituents indicated, especially with reference to the constitution of some of the crystalline substances that should appear in the resins from CIMICIFUGA RACEMOSA.

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